

WE CLAIM:

1. A method of modifying a target site of a gene-of-interest comprising:

a. providing an oligonucleotide that encodes a modification of a gene-of-interest,

5 b. providing a duplex DNA molecule containing said gene-of-interest operably linked to a promoter so that said gene of interest can be expressed in a host organism,

c. providing a cell-free enzyme mixture comprising recombination and gene repair activities and a mismatch repair activity;

10 d. reacting said oligonucleotide, said duplex DNA molecule, and said cell-free enzyme mixture whereby said gene-of-interest is modified at said target site to form a modified gene of interest;

e. introducing said modified gene-of-interest into said host organism; and

15 f. detecting the expression of said modified gene-of-interest.

2. The method of claim 1, wherein said oligonucleotide comprises at least 20 and less than or equal to 200 nucleotides.

3. The method of claim 1, wherein said oligonucleotide comprises at least 10 and less than or equal to 100 Watson-Crick nucleotide pairs.

4. The method of claim 1, wherein said oligonucleotide comprises a single 3' end and a single 5' end.

5. The method of claim 1, 2, 3 or 4, wherein said expression of said modified gene-of-interest confers a selectable trait on said organism.

25 6. The method of claim 1, 2, 3 or 4, wherein said expression of said modified gene-of-interest confers an observable trait on said organism.

7. A method of altering a DNA sequence comprising:

a. providing an oligonucleotide that encodes a

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modification of a DNA sequence,

30 b. providing a duplex DNA molecule containing said DNA sequence,

c. providing a cell-free enzyme mixture comprising recombination and gene repair activities and a mismatch repair activity,

d. reacting said oligonucleotide, said duplex DNA

35 molecule and said cell-free enzyme mixture comprising said recombination and gene repair activities and a mismatch repair activity, whereby said DNA sequence is modified to form an altered DNA sequence, and

e. detecting said altered DNA sequence.

8. The method of claim 7, further comprising fractionating a cell-

40 free composition so as to enrich said altered DNA sequence relative to said DNA sequence, prior to detecting said altered DNA sequence.

9. The method of claim 7 or 8, wherein said oligonucleotide comprises at least 20 and less than or equal to 200 nucleotides.

10. The method of claim 7 or 8, wherein said oligonucleotide comprises at least 10 and less than or equal to 100 Watson-Crick nucleotide pairs.

11. The method of claim 7 or 8, wherein said oligonucleotide comprises a single 3' end and a single 5' end.

12. The method of claim 7 or 8, wherein said oligonucleotide is a duplex mutational vector comprising a contiguous single-stranded self-complementary oligonucleotide having a 3' end and a 5' end, wherein said 3' end and said 5' end are juxtaposed and wherein at least five contiguous nucleotides are Watson-Crick base paired, the sequence of said oligonucleotide comprising a template for said modified DNA sequence.

13. A cell-free composition for the modification of a DNA sequence comprising:

a. a duplex DNA containing a target sequence;

b. an oligonucleotide which targets the DNA sequence and

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encodes the modification thereof;

60 c. a cell-free enzyme mixture comprising recombination
and gene repair activities; and
 d. a reaction buffer.

14. The composition of claim 13, wherein said oligonucleotide
comprises at least 20 and less than or equal to 200 nucleotides.

65 15. The composition of claim 13, wherein said oligonucleotide
comprises at least 10 and less than or equal to 100 Watson-Crick nucleotide pairs.

16. The composition of claim 13, wherein said oligonucleotide
comprises a single 3' and a single 5' end.

70 17. The composition of claim 13, wherein said duplex DNA
sequence is a portion of a gene-of-interest that is operably linked to a promoter, so
that said gene-of-interest can be expressed in a host organism.

18. The composition of claim 13, wherein said cell-free enzyme
mixture lacks mismatch repair activity.

19. The composition of claim 18, wherein said recombination and
gene repair activities are provided by a eukaryote-derived enzyme.

75 20. The composition of claim 19, wherein said cell-free enzyme
mixture is a defined enzyme mixture of purified plant, yeast or mammalian
recombination and repair proteins capable of catalyzing gene repair.

21. The composition of claim 19, wherein said cell-free enzyme
mixture is an extract of a eukaryotic cell.

80 22. The composition of claim 21, wherein said cell-free enzyme
mixture is an extract of a plant cell.

23. The composition of claim 13, wherein said cell-free enzyme
mixture further comprises a mismatch repair activity.

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24. The composition of claim 23, wherein said mismatch repair
85 activity is provided by a eukaryote-derived enzyme.

25. The composition of claim 24, wherein said cell-free enzyme is
an extract of a eukaryotic cell.

26. The composition of claim 25, wherein said cell-free enzyme is
an extract of a plant cell.

90 27. The composition of claim 23, wherein said recombination and
gene repair activities are provided by a eukaryote-derived enzyme.

28. The composition of claim 27, wherein said cell-free enzyme
mixture is a eukaryotic cell extract.

95 29. The composition of claim 13, wherein said oligonucleotide is a
duplex mutational vector comprising a contiguous single-stranded self-
complementary oligonucleotide having a 3' end and a 5' end, wherein said 3' end and
said 5' end are juxtaposed and wherein at least five contiguous nucleotides are
Watson-Crick base paired, the sequence of said oligonucleotide comprising a template
for said modified DNA sequence.

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